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NASA TECHNICAL TRANSLATION NASA TT F- 14353

LASER MICROSPECTRAL ANALYSIS OF BIOLOGICAL OBJECTS

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Translation of "Lazeren mikrospektralen analiz na biologichni obyekti", Godishnik na Sofiskiya Universitet, Fizicheskiya Fakultet, Volume 63, 1968-1969(published in 1971), pp. 123-135.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D.C. 20546 AUGUST 1972

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LASER MICROSPECTRAL ANALYSIS OF BIOLOGICAL OBJECTS

[Article by G. Dimitrov et al; Sofia, Godishnik na Sofiskiya Universitet, Fizicheskiya Fakultet, Bulgarian, Volume 63, 1968-1969 (published in 1971), pp 123-135]

As early as 1934 Gerlach noted the potential use of conventional emission spectral analysis in medicine and in biomedical research (1). In spite of this fact, emission spectroscopy methods are not being sufficiently utilized in the investigation of various problems in medicine and biology. Recently scientists have elucidated the enormous role played in the life and development of animals and plants of certain microelements contained in these organisms (2, 3, 4). In determination of small quantities of various inorganic admixtures, emission spectral analysis has proven particularly suitable, due to its simplicity, high degree of sensitivity, speed and reliability.

Analysis of biological products using conventional emission spectroscopy methods involves certain peculiarities in methods of preparing and exciting samples, which in some cases complicate the job. On the other hand, in the majority of cases of analysis of biological objects, conventional local spectral analysis methods are unsuitable. For example, with these methods it is impossible to establish the difference in content of microelements in variously-colored wing membranes of butterflies, to trace the distribution of microelements in a human hair, to investigate the difference in content of inorganic components in the internal and external organs of insects.

It is well known that only a year after the discovery of lasers initial attempts were made at laser microspectral analysis (including local analysis on biological objects). In 1963 researchers at Boston University conducted a number of experiments connected with biochemistry, anatomy and pathology with the aid of a laser microspectral analyzer manufactured by the Dzharel Ash [transliteration] Company (5). We know of several other experiments involving the application of laser microspectral analysis to the investigation of biological objects (6, 7, 8). These also involve investigations connected with the anatomy, pathology, and biochemistry of human and animal organisms.

There are no known published studies dealing with the application of laser microspectral analysis in entomology, that is to determine the quantitative distribution of elements in individual organs and systems in the body of an insect. The small size of insects and the negligibly small quantity of materials forming their organs make it impossible to investigate with conventional local spectral analysis methods. This technique, however, is fully possible with the laser microspectral analyzer, the capabilities of which are quite extensive, making it possible to solve many problems which are impossible to solve with other methods. The subject of this article is: investigations connected with the establishment of a link between pigmentation and the presence of metallic components and their role in the coloration of butterflies: *Arginis latonia, Pyrameis cardui, Vanessa Jo*, investigation of the distribution of certain inorganic components in the external and internal organs of the common housefly, *Musca domestica*, and establishment of the presence and distribution of metallic components in human teeth and hair.

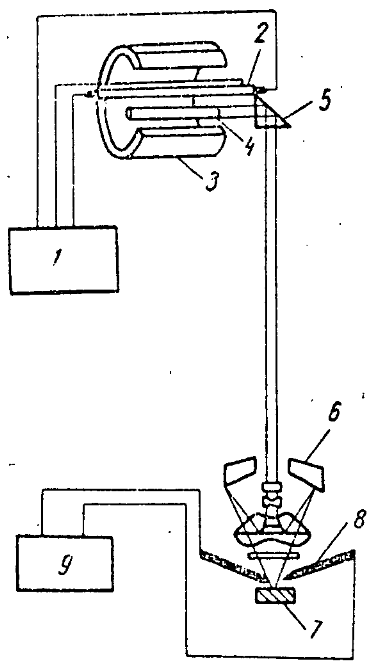

Figure 1. Diagram of LMA-1 laser microspectral analyzer

Figure 1 contains a diagram of the LMA-1 laser spectral analyzer which was used in our experiments; this instrument was manufactured in the GDR by Carl Zeiss Jena (9). Block 1 powers pulse xenon flash lamp 2, which is located at one of the focal points of elliptical mirror 3 and serves to excite the generation of laser radiation. Active body 4 is located at the elliptical mirror's second focal point; this body may be a ruby crystal or neodymium-activated glass. The LMA-1 microspectral analyzer uses neodymium glass, which produces laser radiation with a wavelength of 1060 nm. The laser beam, with the aid of full internal reflection prism 5, changes its direction by 90° and strikes special microscopic lens 6. When the laser equipment is being used, two lenses, which can be quickly changed, are mounted above an easily movable optical track. The working distances of the two lenses are much greater than those of conventional microscope lenses. It is 14 mm for the 16 x /0.20 lens objective, and 15.8 mm for the 40 x /0.5 mirror-lens objective. In order to protect the objectives from injury during the vaporization of specimens, protective glass plates are mounted in front of them. These objectives, in addition to focusing the laser beam, are used for observing the specimen, for selecting the area from which the sample is to be vaporized, and for photographing the specimen after it has been acted upon by the laser beam. For this purpose additional devices are mounted on the laser equipment: eyepieces, polarization devices, compensation plates and a lighting system. The laser beam is focused on specimen 7, from which matter explosively vaporizes. The light emission of the obtained microplasm is not sufficient to register on a spectral photographic plate. It is subjected to additional excitation with the aid of tapered-end electrodes 8 of spectrally pure carbon, which are connected to capacitor battery 9, which charges to a specified voltage prior to pulse discharge. The parameters of the discharge circuit -- capacity and self-induction -- are selected in conformity with the type of specimen and components being examined. The obtained microplasm comes between the electrodes and causes a pulse discharge between the two electrodes, as a consequence of which the spectrum of the vaporized substance is excited. With the aid of a single-lens system the light emission of the microplasm is projected onto the slot of a Q-24 or PGS 2 spectrograph. The spectrum is photographed with suitable type Blau Hart or Blau Rapid spectral photographic plates and processed in special Phenidone developers (10, 11, 12).

The laser microspectral analyzer provides very sharp laser beam focusing, as a result of which a large quantity of energy is concentrated on a small area of the specimen. With laser equipment, localization capability is extremely good. With the changeable lenses and a suitable selection of pulse lamp power supply parameters, and with a special optical microdevice with three interchangeable blinds, it is possible to vaporize a sample from a spot ranging from 10 to 300 μm in diameter and a quantity of matter in the order of 10^{-6}g. The shape and type of crater obtained following laser action depend substantilaly on the specimen. Figure 2 contains craters obtained by laser beam action on: a) steel; b) galena; c) a chondrule from a meteorite; d) tooth.

It has been established from past investigations of insect pigmentation that this pigmentation is connected with certain organic pigments located on the body surface. In the past no definite proof has been obtained on the role of inorganic components in the forming of specific colors. In butterflies these pigments are found in membrane plates, typical of the order, situated in tegular fashion on both sides of the wings. The great variety of colors characteristic of this order is due to the combination of differently pigmented membrane plates as well as the optical properties of

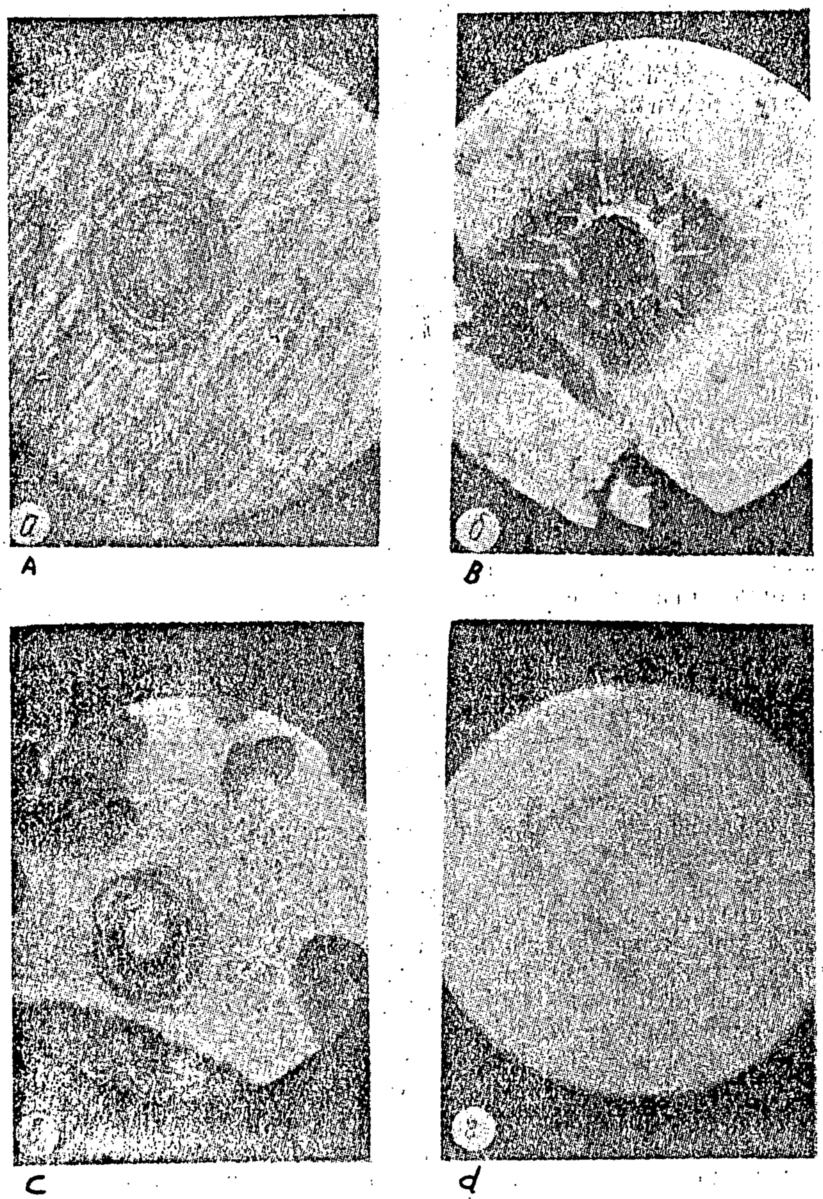

Figure 2. Photomicrographs of laser effect on

a -- steel; b -- galena; c -- chondrule from a meteorite; d -- tooth

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some of these. In the past membrane plates of interest would be removed from the butterfly wing with the aid of a glass needle under magnification and would be placed on backings of spectrally pure charcoal, in order to avoid vaporization of specimen from the platelets on the underside of the wing. It was later established that this influence is negligible and that the analysis can be made directly, firing directly at the selected wing platelets. The spectra were obtained as a result of two laser pulses. It is possible to vaporize a specimen of single platelets. Figure 3 contains photomicrographs of individual platelets from a butterfly wing: a -- before laser effect, and b -- after laser effect. Figure 4 shows the tegular arrangement of platelets in a butterfly wing: a -- before laser effect, and b -- after laser effect. It is evident from the two figures that with the No 3 blind of the optical microdevice of the LMA-1 laser microspectral analyzer, laser action does not destroy the entire platelet nor the wing. This makes it possible to conduct the analyses on differently pigmented platelets without removing the butterfly wing and without affecting neighboring platelets.

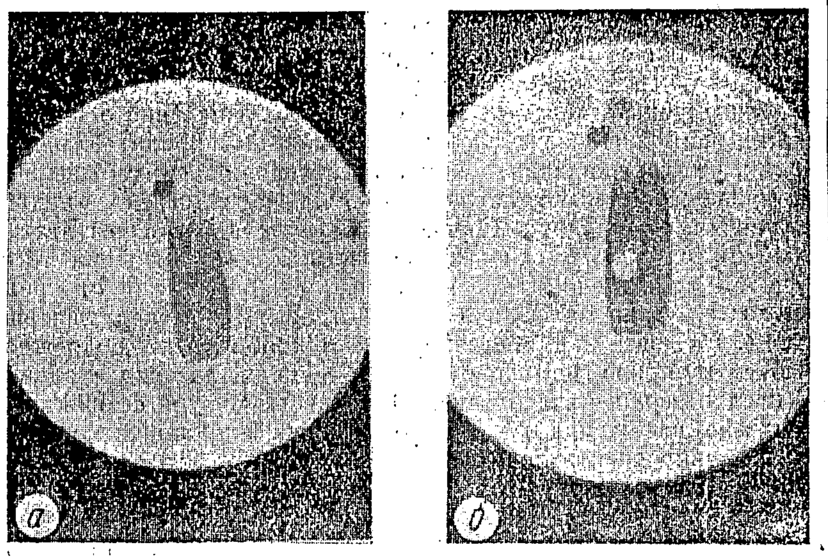

Figure 3. Photomicrograph of individual membrane platelet of a butterfly wing:

a -- before, and b — after laser effect

The presence of of the following inorganic components was established in the investigated butterfly species: Fe, Si, Mn, Al, Ca, Cu, Ti, B. The concentration of these microelements differed in the differently-pigmented platelets. Table I contains relative intensities of elements in differently pigmented platelets. This difference cannot be caused by contaminants, since it would not possess a selective property for some of the colors. Consequently one can assume that inorganic components play a certain role in butterfly pigmentation.

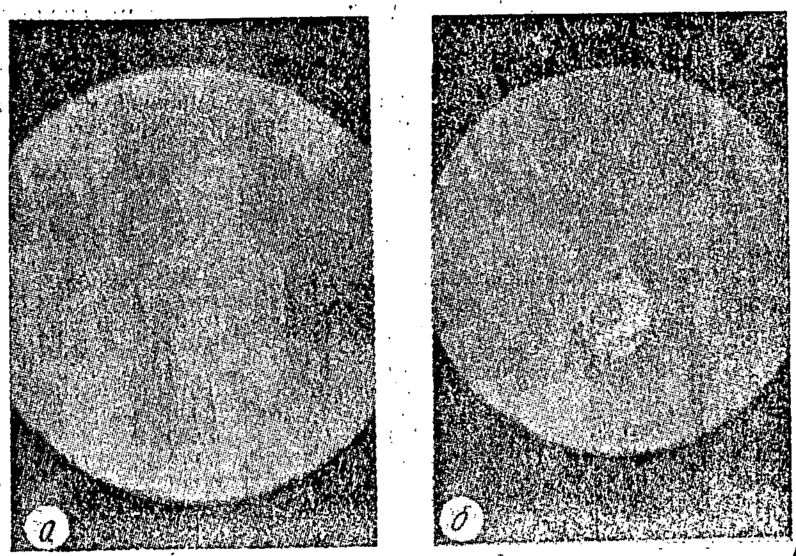

Figure 4. Photomicrographs of tegular-arranged membrane platelet on butterfly wing:

a -- before, and b -- after laser effect

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1 Спектрална линия \ 2 Цвят на плочките			3 Бял	4 Червен	5 Жълт	6 Черен	7 Розов
Ti	II	3349,04	0,450	0,310	0,220	0,186	0,170
Fe	II	2598,37	0,070	0,170	0,072	0,164	0,064
Ca	II	3158,89	0,520	0,170	0,480	0;940	0,220
B	II	2796,78	0,133	0,125	0,316	0,360	0,144
Mg	II	2795,53	1,540	1,780	1,450	1,780	1,520
Si	I	2881,70	0,400	0,980	0,600	0,880	0,560
Cu	I	3247,54	0,340	0,350	0,570	0,730	0,320
Mn	II	2576,10	0,073	0,200	0,118	0,160	0,140
Al	I	3082,16	0,106	0,250	0,200	0,180	0,178

Table I. Relative Intensities of Spectral Lines of Metallic Components in Differently-Pigmented Platelets from the Wings of the *Arginis latonia* butterfly

Key to table: 1 -- spectral lines; 2 -- color of platelets; 3 -- white; 4 -- red; 5 -- yellow; 6 -- black; 7 -- pink

(Translator's Note: Beginning with this table all commas are equivalent to decimal points.)

1 Елементи	2 Черен			3 Червен			4 Бял		
	I	II	III	I	II	III	I	II	III
Fe	0,144	0,126	0,130	0,170	0,154	0,174	0,070	0,072	0,130
Si	0,880	0,780	0,710	0,980	0,843	0,900	0,400	0,480	0,460
Mn	0,184	0,210	0,182	0,200	0,184	0,206	0,073	0,089	0,117
Mg	1,780	1,820	1,750	1,780	1,800	1,810	1,450	1,380	1,530
Al	0,220	0,220	0,160	0,250	0,210	0,182	0,106	0,134	0,091
Ca	0,940	0,870	0,920	1,170	1,210	1,290	0,520	0,580	0,620
Cu	0,730	0,750	0,724	0,350	0,460	0,390	0,340	0,250	0,270
Ti	0,186	0,160	0,162	0,310	0,258	0,286	0,450	0,333	0,420
B	0,360	0,310	0,252	0,125	0,093	0,107	0,133	0,117	0,095

Table II. Relative Intensities of Spectral Lines of Metallic Components Discovered in Identically Pigmented Platelets from the Wings of the Following Butterflies: I *Arginis latonia*, II *Pyrameis cardui*, III *Vanessa Jo* Collected at the Same Site

Key to table: 1 -- elements; 2 -- black; 3 -- red; 4 -- white

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Table II contains data on the relative distribution of metallic components discovered in identically-pigmented platelets from the wings of three species of butterfly collected at the same site. It is apparent that in spite of the species differences of these butterflies, a large portion of the metallic components are present in relatively the same quantities. This shows that the metal components are characteristic not of species but rather of pigment.

1 Цвят на плочките	Fe II 2598,37		Al I 3082,16		Cu I 3247,54	
	I	II	I	II	I	II
2 Червен	0,072	0,168	0,104	0,240	0,167	0,440
3 Черен	0,084	0,152	0,118	0,180	0,148	0,255
4 Розов	0,052	0,105	0,070	0,162	0,135	0,420
5 Бял	0,094	0,145	0,103	0,210	0,190	0,370
6 Златист	0,050	0,093	0,094	0,128	0,175	0,440

Table III. Relative Intensities of Spectral Lines of Iron, Aluminum and Copper Contained in Differently-Pigmented Platelets from the Wings of the *Vanessa Jo* butterfly. I -- Specimens Collected at a Site Far From Industrial Plants; II -- Specimens at a Site Close to Industrial Plants

Key to table: 1 -- color of platelets; 2 -- red; 3 -- black; 4 -- pink; 5 -- white; 6 -- gold

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A large number of butterflies from various sites were examined: mountain areas far from industrial plants, where one can assume an absence of industrial pollution, and areas in the vicinity of industrial plants. Table III contains the results of our investigation; it contains figures on the relative distribution of iron, copper, and aluminum in two specimens of the butterfly *Vanessa Jo*. It is obvious that these elements are present in larger quantities in the specimens collected close to an industrial plant.

The presence of varying quantities of metallic components in differently-pigmented sectors of butterfly wings gives us reason to assume that these components play a certain role in the forming of the corresponding pigments. This is bolstered by the fact that the quantity of a large number of metallic components is relatively close in identically-pigmented platelets of various butterfly species. Complete elucidation of this question will require additional physiological and ecological investigations. The effect of industrial dust particles in the air is also an interesting fact which merits attention. Complete clarification of this matter will be the aim of an investigation of samples which will be handled entirely under laboratory conditions. The utilized method essentially makes it possible to study a number of patterns pertaining to insect biology: habitat, migration, etc. At the same time another method is available for studying the distribution of industrial and other pollution around industrial plants and populated areas.

The object of the second group of investigations in this study is the common housefly, *Musca domestica*. It is a member of the group of synanthropic insects. Its development and life are intimately linked with that of man, as a consequence of which it is an object of comprehensive investigation. Particularly important are investigations connected with the capability of tolerating substances which are biologically and mechanically harmful to man.

The insect specimens were examined in the laboratory and were the offspring of t h e s a me parents. This eliminated the possibility of any racial or population differences in the investigated individuals. Examination of the insects in an identical environment as well as under identical ecological conditions means that contaminants will be present to an identical degree in all specimens.

Table IV lists the elements discovered in certain external organs of the housefly: wing, proboscis, antennae, and t h e three pairs of extremities, that is those organs which come into contact most actively with the external environment. Examinations of the extremities were made on the last tarsal member. It is evident from the table that the distribution of metallic components is rather varied in the selected organs. In addition to the elements detected in the wings of the butterflies, the flies contain the elements zinc and phosphorus. From a comparison of data on the wings, antennae, and proboscis, the spectral lines of the detected elements in the wings show the greatest constancy of r e la t i v e intensity, from which we can conclude that of the three examined organs, metal components are present in the largest quantities in the wings, with the exception of zinc, while presence of a large number of the detected elements is approximately the same for the antennae and

1 Спектрална линия	2 Крилe	3 Хобот	4 Антени	5 Крайници		
				I чифт 6	II чифт	III чифт
Fe II 2599,39	0,772	0,249	0,139	0,590	0,503	0,743
Si I 2516,11	0,731	0,230	0,278	0,455	0,488	0,650
Mn II 2593,73	0,182	0,018	0,062	0,074	0,132	0,196
Al I 3092,72	0,400	0,150	0,175	0,307	0,278	0,410
Cu I 3273,96	0,219	0,195	0,151	0,216	0,183	0,250
Ti II 3372,80	0,079	0,050	0,048	0,036	0,071	0,162
B I 2497,73	0,550	0,432	0,331	0,511	0,485	0,600
Zn I 3345,02	0,069	0,111	0,018	0,074	0,334	0,071
Cr II 3158,87	0,800	0,331	0,490	0,476	0,520	0,665
Mg II 2802,69	1,310	1,220	1,490	1,216	1,370	1,330
P I 2535,65	0,127	0,080	0,088	0,127	0,184	0,138

Table IV. Relative Intensities of Spectral Lines of Elements Discovered in Certain External Organs of *Musca domestica*

Key to table: 1 -- spectral line; 2 -- wing; 3 -- proboscis; 4 -- antennae; 5 -- extremities; 6 -- pair

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proboscis. This can be explained to a certain degree by the fact that they are in the immediate vicinity and frequently come into contact with one another. We might draw attention to the extremities. The three pairs of extremities are similar organs in origin and function, and one cannot assume that there will be a difference in composition. In spite of this fact it is apparent from the table that the presence of the detected elements is greatest in the third pair of extremities, with the exception of zinc and phosphorus. We know from observation that the third pair of extremities performs the most active movement; this is most probably the reason for the presence of the largest quantities of the detected elements, due to contaminants.

1 Спектрална линия	2 Церебрални ганглии	3 Яйчници	4 Гръдна мускулатура
Fe II 2599,39	0,371	0,356	0,419
Si I 2516,11	0,328	0,308	0,425
Mn II 2593,73	0,046	0,028	0,018
Mg II 2802,69	1,530	0,910	1,155
Al I 3092,71	0,233	0,173	0,260
Ca II 3179,33	0,740	0,278	0,375
Zn I 3345,07	0,090	0,000	0,021
Ti II 3349,04	0,051	0,070	0,105
B I 2497,73	0,132	0,258	0,452
P I 2535,65	0,074	0,168	0,166

Table V. Relative Intensities of Spectral Lines of Elements Detected in Certain Internal Organs of *Musca domestica*

Key to table: 1 -- spectral line; 2 -- cerebral ganglia; 3 -- ovaries; 4 -- thoracic musculature

Table V contains data on the investigation of internal organs of the common housefly. Since the three compared organs are internal, difference in metallic component content is due solely to difference in the chemical composition of the tissue forming them. It is also apparent that zinc is not present in the ovaries.

Another group of investigations was made to determine the distribution of microelements along a human hair 2 to 2.5 cm in length. The hair was successively laser-beam treated from root to end every 1 mm. In view of the minimal content of microelements, every spectrum was obtained from two laser pulses. Thanks to the extreme localization capability of the laser device, it was possible to obtain vaporization of specimens from small areas of the pair without the hair being destroyed. Figure 5: a, b, c shows three hair sections following laser treatment, of root, middle, and hair end respectively. We established the presence of the elements Ca, Mg, Si, Cu, Al, Ti in the hair. It is interesting to note that the distribution of microelements is not uniform along the hair. Presence of copper and aluminum diminishes from the root toward the end. This fact is probably linked with hair aging.

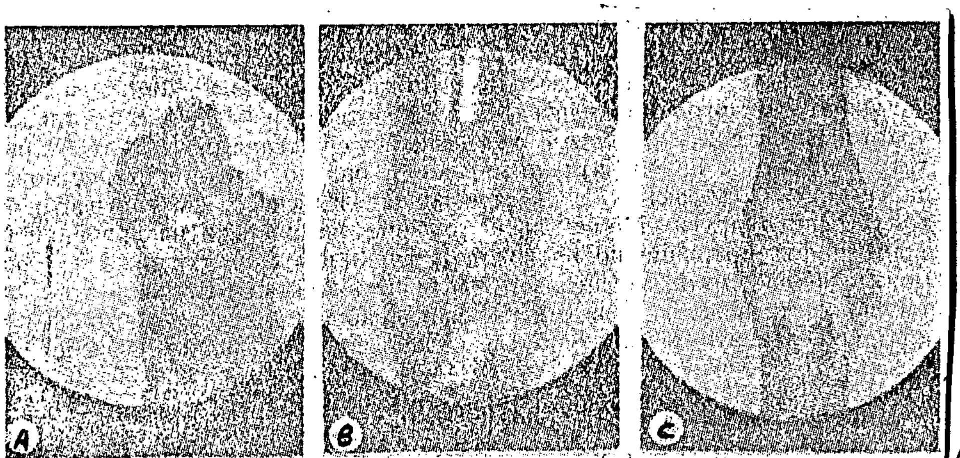

Figure 5. Photomicrograph of human hair following laser treatment:

a -- root; b -- middle; c -- hair end

We also made some preliminary experiments to establish the presence and distribution of inorganic components in several regions of a human tooth. We established the presence of the elements Mg, Al, Si, Ca, P, Cu, Zn in three regions: dental calculus above the gum line, calculus below the gum line, and in the bony tissue proper. Calcium content in the dental calculus is much greater than in the bony tissue. Aluminum was present in negligibly small quantities only in the dental calculus above the gum line. Zinc and phosphorus are present in minimum quantities in the bony tissue, while larger quantities are present in dental calculus. Figure 2 d shows a laser-produced crater on a tooth. A comparative study of distribution of micro-elements in deciduous and permanent teeth remains for future investigation.

We can draw the following conclusions from this study:

1. The presence of inorganic components in the examined biological objects has been established.

2. Inorganic components play a certain role in the pigmentation of butterfly wings.

3. Metallic components are characteristic of pigment and not species.

4. With the method utilized it is possible to investigate the distribution of industrial and other pollution in the vicinity of populated localities and industrial plants.

5. It is possible to study a number of patterns in the biology of habitat, migration, etc of insects with this method.

6. Utilization of the LMA-1 laser microspectral analyzer to investigate biological objects is extremely promising and can shed light on the distribution of many microelements which are vitally important for plants and animals.

7. Spectral investigations on a time axis involving laser microspectral analysis of biological objects are extremely promising and present exceptional interest from both a physical and biological standpoint.

8. Studies should be of the distribution of microelements in the process of food injection through the digestive tract of insects and their larvae.

Submitted 17 February 1970

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